Analysis of endoplasmic reticulum trafficking signals by combinatorial screening in mammalian cells

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To address issues of quality control during ion channel assembly, we have studied the assembly-dependent trafficking of adenosine 5′-triphosphate (ATP)-sensitive K^+ channels (K_{ATP}). These channels couple the metabolic state of the cell to membrane excitability. K_{ATP} channels have an unusual octameric stoichiometry consisting of four pore-lining inward rectifier a a subunits (Kir6.1/6.2; two transmembrane segments) like other K^+ channels, but also containing four regulatory sulphonylurea-binding b subunits (SUR1/2A/2B; probably seventeen transmembrane segments) that belong to the ATP-binding cassette (ABC) family of proteins.

We found that only octameric K_{ATP} channel complexes were capable of expressing on the cell surface, implying that quality control mechanisms must exist to prevent monomers and partial complexes from expressing on the cell surface. Surprisingly, the primary quality control mechanism during K_{ATP} assembly did not involve endoplasmic reticulum (ER) degradation or ER chaperones, but rather the exposure of an ER retention/retrieval signal (RKR) present in cytosolic domains of each subunit. Mutating the retention sequences did not affect protein levels, but allowed surface expression of monomers and partially assembled complexes, including improperly gated channel combinations. Interestingly, this RKR motif did not require proximity to the N- or C-terminus like all other known ER retention/retrieval signals, and therefore may be more common. In conclusion, quality control during KATP assembly is mediated by a short trafficking signal whose exposure reflects the assembly state of the channel. These results provide a clear example of how a trafficking checkpoint serves as an important quality control mechanism during the assembly of an ion channel complex. Furthermore, they identify a new ER retention/retrieval motif that could explain transient or permanent ER localization of many proteins.

To improve the accuracy of predicting arginine-based sorting signals, we developed a general methodology for defining trafficking signal consensus sequences. Our approach utilizes retroviral gene transfer to create combinatorial expression libraries of trafficking signal variants in mammalian cells, flow cytometry to sort cells based on trafficking phenotype, and quantitative trafficking assays to measure the efficacy of individual signals. Using this strategy to analyze arginine- and lysine-based ER localization signals, we demonstrate that small changes in the local sequence context dramatically alter signal strength, generating a broad spectrum of trafficking phenotypes. Finally, using sequences from our screen, the potency of di-lysine, but not di-arginine, mediated ER localization was correlated with the strength of interaction with α-cystein-rich modular protein.

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